

## Prolonged fecal shedding of Shiga toxin-producing *Escherichia coli* among children attending day-care centers in Argentina

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### ABSTRACT

In this report we describe the detection and duration of fecal shedding of Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 in symptomatic and asymptomatic cases during four events occurred among children in day-care centers in Argentina. In each event, the cases were identified among children, family contacts and staff members of the Institution. The isolates were characterized by pheno-genotyping and subtyping methods. The STEC fecal shedding was prolonged and intermittent. Strains O157:H7 (1<sup>st</sup> event); O26:H11 (2<sup>nd</sup> event); O26:H11 (3<sup>rd</sup> event) and O145:NM (4<sup>th</sup> event) were shed during 23-30, 37, 31 and 19 days, respectively. Considering the possibility of STEC intermittent long-term shedding, symptomatic and asymptomatic individuals should be excluded from the Institution until two consecutive stool cultures obtained at least 48 h apart, test negative.

**Key words:** Shiga toxin-producing *Escherichia coli*, prolonged fecal shedding, children day-care centers

### RESUMEN

**Excreción prolongada de *Escherichia coli* productor de toxina Shiga en niños que concurren a jardines maternos de Argentina.** En el presente trabajo se describe la detección y el tiempo de excreción de *Escherichia coli* productor de toxina Shiga (STEC) O157 y no-O157 en casos sintomáticos y asintomáticos durante cuatro eventos ocurridos en jardines maternos de Argentina. En cada evento se identificaron los casos entre los niños, sus familiares y el personal del jardín. Los aislamientos fueron caracterizados por técnicas feno-genotípicas y de subtipificación. La excreción de STEC fue, en general, prolongada e intermitente. Cepas STEC O157:H7 (1<sup>er</sup> evento); O26:H11 (2<sup>do</sup> evento); O26:H11 (3<sup>er</sup> evento) y O145:NM (4<sup>to</sup> evento) fueron excretadas durante 23-30, 37, 31 y 19 días, respectivamente. Dadas las características de la excreción, no debe permitirse el reingreso a la institución de todo niño o adulto con infección por STEC, sintomático o asintomático, hasta no tener dos coprocultivos negativos sucesivos, con intervalos de 48 horas entre ellos.

**Palabras claves:** *Escherichia coli* productor de toxina Shiga, excreción prolongada, jardines maternos

Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 strains have been associated with outbreaks and sporadic cases of human disease, ranging from uncomplicated diarrhea to hemorrhagic colitis and hemolytic uremic syndrome (HUS). STEC is transmitted to humans through contaminated food, water, and direct contact with infected persons or animals (1). Person-to-person transmission is well documented in day-care and extended-care facilities, most likely because of the low infection dose and the increased susceptibility of the individuals in these facilities (4). Shedding of *E. coli* O157 can be prolonged and intermittent (12). In contrast, little information about STEC non-O157 excretion is available. The aim of this study was to describe the detection and duration of fecal shedding of STEC O157 and non-O157

in symptomatic and asymptomatic cases during four events occurred among children in day-care centers. Rectal swabs were collected and routinely cultured for intestinal pathogens at the Regional Microbiological Laboratories. Sorbitol-MacConkey agar plates were sent to the National Reference Laboratory (NRL) for screening purposes. The STEC strains were isolated and characterized as described elsewhere (8, 10). The duration of STEC shedding was defined as the interval between the onset of diarrhea and the last positive sample followed by two consecutive negative stool cultures obtained at least 48 h apart.

1<sup>st</sup> Event: In November 2002, a 12-month-old girl, attending a day-care center in Buenos Aires City, had bloody diarrhea associated with *E. coli* O157:H7. A study was

conducted to determine the *E. coli* O157 spreading among 11 children, 3 staff members, and 6 household contacts. Only one positive-asymptomatic five-month-old boy, attending the same room, was identified. *E. coli* O157:H7 strains isolated from both children carried: *stx*<sub>2c-O157-EDL933</sub> + *stx*<sub>2c-O157-E32511</sub>, LEE-encoded intimin (*eae*) gene and enterohemolysin (*ehxA*) genes, belonged to phage type (PT) 47, and showed the same profile by *Xba*I-pulsed-field gel electrophoresis (*Xba*I-PFGE). The shedding was prolonged and intermittent in both children. The symptomatic child carried the organism for 23 days and the asymptomatic child for 30 days respectively.

2<sup>nd</sup> Event: From October 15 to November 8, 2003, a gastrointestinal outbreak occurred at a day-care center in Mar del Plata City. Fourteen (17.5%) out of 80 children, and one of the children's mother had diarrhea (3). One case developed HUS. A STEC O26:H11 strain in a 9-month-old boy with uncomplicated diarrhea, and a STEC O103:H2 strain in another 11-month-old boy were identified. Both isolates were positive for *stx*<sub>1-O157-EDL933</sub>, *eae* and *ehxA* genes. The child with the STEC O26:H11 infection shed the pathogen for 37 days, whereas the child with the STEC O103:H2 infection had a shorter excretion because fecal samples taken at 15 and 29 days following the onset of symptoms were STEC-negative. Although STEC strains were isolated in two children, they were not associated as etiologic agents of the outbreak.

3<sup>rd</sup> Event: Between January 23 and 31, 2004, an outbreak of bloody diarrhea occurred at a day-care center in Paraná City (5). Four (10%) out of 40 children presented bloody diarrhea and one case developed HUS. All children, 5 staff members, and six family contacts of cases were screened for STEC infection. *E. coli* O157:H7 was isolated from three bloody diarrhea cases and one asymptomatic child. The isolates were characterized as *stx*<sub>2-O157-EDL933</sub> + *stx*<sub>2c-O157-E32511</sub>, *eae*, *ehxA*, and PT4, and showed an indistinguishable *Xba*I-PFGE pattern. In another asymptomatic child, a PCR-*stx*<sub>2</sub>/*rfb*O157 positive sample was identified, without isolation. One asymptomatic child with *E. coli* O26:H11, *stx*<sub>1-O157-EDL933</sub>, *eae*, *ehxA* infection continued shedding the organism for 31 days. All specimens from the staff and family contacts were negative.

4<sup>th</sup> Event: On January 6, 2005, four HUS cases occurred at a day-care center in Rosario City. Stool samples from attendees, staff members of the Institution and family contacts of confirmed positive-STECS cases were collected and analyzed. At the NRL, 3 (5%) of 53 samples were confirmed as STEC-positive. An infection associated with STEC O145:NM, *stx*<sub>2-O157-EDL933</sub>, *eae*, and *ehxA* was confirmed in a 12-month-old-boy and a 24-month-old-girl. An STEC ONT:HNT, *Stx*2d2-O91/b-B2F1, *eae*, and *ehxA* was recovered from an asymptomatic teacher. The O145:NM positive-boy continued to shed the organism for 18 days, but during the fifth control, an association with a ONT:HNT STEC strain with the same genotypic profile as the teacher's strain, was detected.

The O145:NM positive-girl and the ONT:HNT positive-teacher, shed the strains for 19 days. All the O145:NM strains showed the same *Xba*I-PFGE pattern. All specimens from family contacts were negative.

This report showed the long-term shedding of STEC O157 and non-O157 during day-care-associated outbreaks. Shah *et al.* reported a median duration of O157 shedding of 29 days (range, 11-57) during a day-care-associated outbreak (11). A study performed in Germany, revealed a median duration of O157 shedding of 13 days (range, 2-62) and 21 days (range, 5-124) in diarrhea and HUS patients, respectively (6). To our knowledge, this is one of the first reports of non-O157 long term shedding in humans. It is important to remark that *E. coli* O145:NM and O26:H11 have been associated with severe human disease worldwide and they are the second and third most common serotypes isolated in Argentina, respectively (9). Similarly to Gouveia *et al.* (4), we recommended that in the event of day-care center outbreaks, asymptomatic children and personnel should be tested because they may also be infected and shedding this pathogen despite the absence of symptoms. We also emphasize that symptomatic and asymptomatic children and staff members excreting STEC should not be allowed to return to the day-care facilities as long as they continue to shed this pathogen. Because of the possibility of STEC intermittent long-term shedding, the standard for no infectivity might require two negative stool samples collected at 2-day interval. The study by Belongia *et al.* (2) has shown that there was no evidence of person-to-person transmission at day-care facilities when children with diarrhea were allowed to return to the facility after two consecutive O157-negative stool cultures. Moreover, individuals with diarrhea illness should be excluded from the center until symptoms resolve whether or not a pathogen is identified. These four events emphasize the importance of prompt case reporting, as well as follow-up testing for the pathogen in stool culture. In this study, a multiplex-PCR for *stx*1 / *stx*2 / *rfb*O157 gene detection, a sensitive and specific technique for screening of both O157 and non-O157 STEC strains, was included (7). Day-care centers are institutions where hygiene practices are difficult to ensure. It is important to consider that routine hand washing is one of the best ways to prevent the spread of infection among the children. Considering that the use of the swimming pool was identified as the only risk factor epidemiologically linked to the 3<sup>rd</sup> event (OR, 10.0; 95% CI, 1.3-77.0) (5), efforts to educate pool owners and parents on the importance of proper pool maintenance with continuous chlorination, are necessary to improve safety in this type of activities. The management of these events highlighted both the long-term STEC shedding and the risks posed by child care facilities in the pathogen transmission. The knowledge of transmission routes and vehicles will allow professional health workers, teachers and parents to be

educated on reducing risky behavior, which can decrease their risk for STEC infection.

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## REFERENCES

1. Armstrong G, Hollingsworth J, Morris G. Emergin foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of new pathogen into the food supply of the developed world. *Epidemiologic Reviews* 1996; 18: 29-51.
2. Belongia E, Osterholm M, Soler J, Ammend D, Braun J. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. *JAMA* 1993; 269: 883-8.
3. Gomez D, Miliwebsky E, Silva A, Deza N, Zotta C, Cotella O, *et al.* Aislamiento de *Escherichia coli* productor de toxina Shiga durante un brote de gastroenteritis en un Jardín Maternal de la Ciudad de Mar del Plata. *Rev Argent Microbiol* 2005; 37:176-83.
4. Gouveia S, Proctor M, Lee M, Luchansky J. Genomic comparisons and Shiga toxin production among *Escherichia coli* O157:H7 from a day care center outbreak and sporadic cases in southeastern Wisconsin. *J Clin Microbiol* 1998; 36: 727-33.
5. Ilardo M, Agüero G, Chinen I, Miliwebsky E, Pedroni E, Sosa-Estani S, *et al.* Brote de diarrea por *Escherichia coli* O157:H7 en un Jardín Maternal. Paraná-Entre Ríos, Enero 2004. Congreso Latinoamericano y X Congreso Argentino de Microbiología, 2004, Resumen 643, Buenos Aires, Argentina.
6. Karch H, Russman H, Schmidt H. Long-term shedding and clonal turnover of enterohemorrhagic *Escherichia coli* O157 in diarrheal diseases. *J Clin Microbiol* 1995; 33: 1602-5.
7. Leotta GA, Chinen I, Epszteyn S, Miliwebsky E, Melamed IC, Motter M, *et al.* Validación de una técnica de PCR multiple para la detección de *Escherichia coli* productor de toxina Shiga. *Rev Argent Microbiol* 2005; 37: 1-10.
8. Rivas M, Caletti MG, Chinen I, Refi SM, Roldán CD, Chillemi G *et al.* Home-prepared hamburger and sporadic hemolytic uremic syn-drome, Argentina. *Emerg Infect Dis* 2003; 9: 1184-6.
9. Rivas M. Control and prevention of Shiga toxin-producing *Escherichia coli* infections in humans and in the animal reservoir. II Simposio Internacional de Biotecnología, 2004, Conferencia C5, p. 5, Tucumán, Argentina.
10. Scheutz F, Beutin L, Pierard D, Smith R. Nomenclature of verocytotoxins. In: Garvey P, McDowell DA, editors. *Verocytotoxigenic E. coli*. Trumbull, Connecticut, USA. Food & Nutrition Press, 2001, p 447-52.
11. Shash S, Hoffman R, Shillam P, Wilson B. Prolonged fecal shedding of *Escherichia coli* O157:H7 during an outbreak at day care center. *Clin Infect Dis* 1996; 23:835-6.
12. Swerdlow DL, Griffin PM. Duration of faecal shedding of *Escherichia coli* O157:H7 among children in day-care centres. *Lancet* 1997; 349: 745-6.